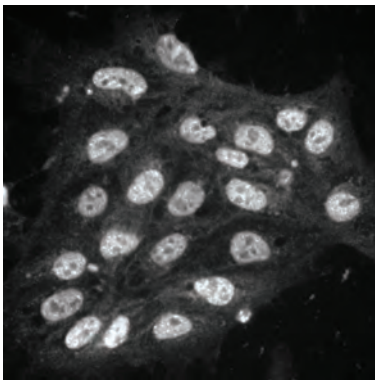
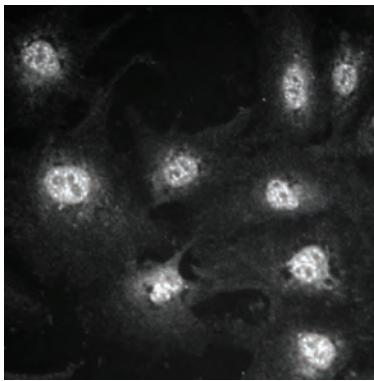


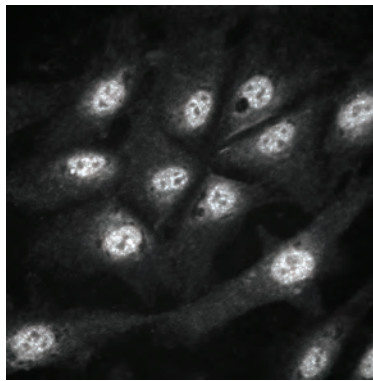
Conditioned
Media



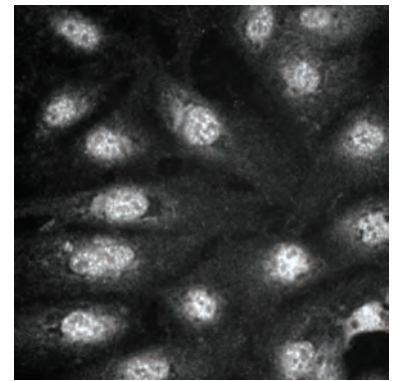
KSR
+ Noggin



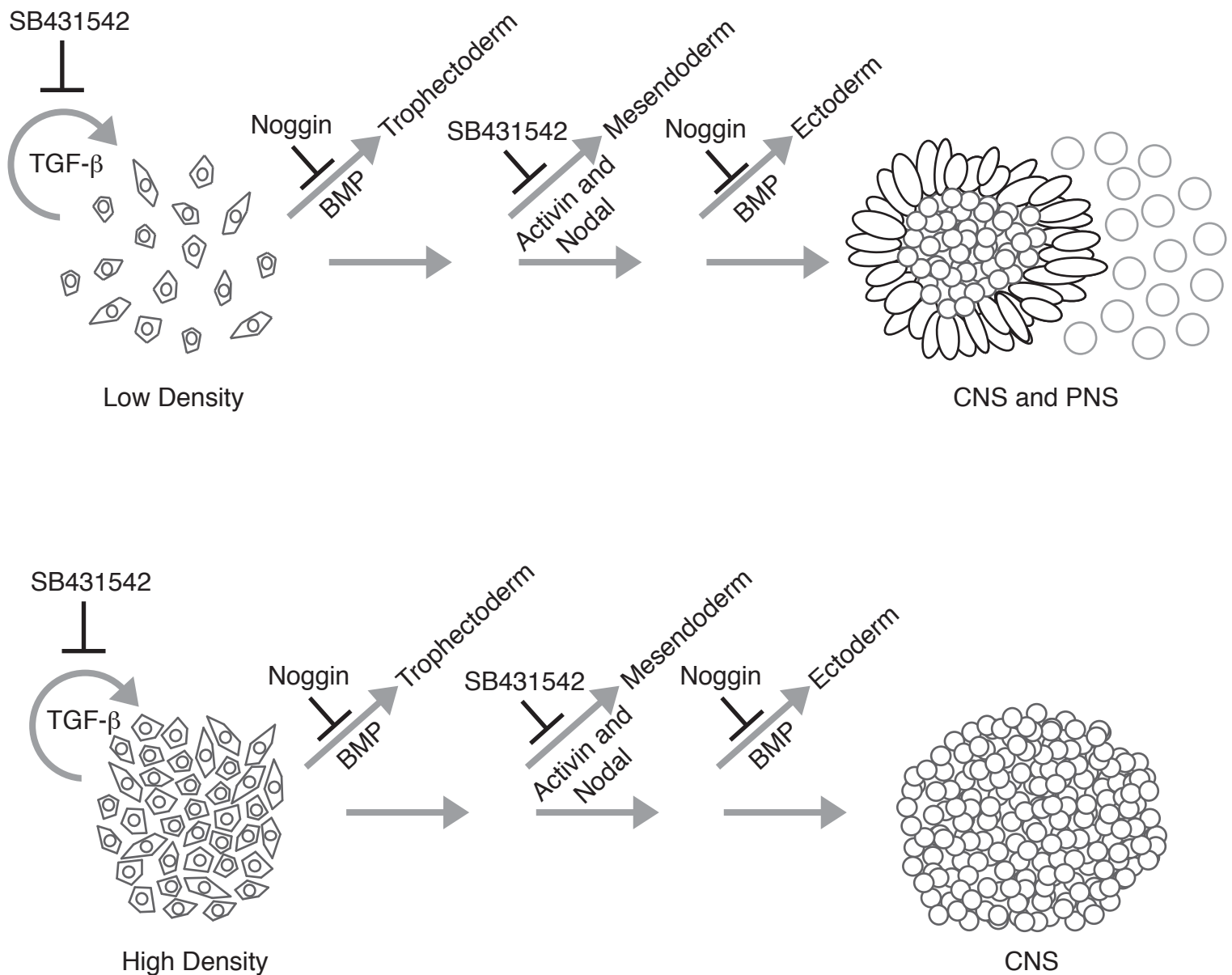
KSR
+ SB431542



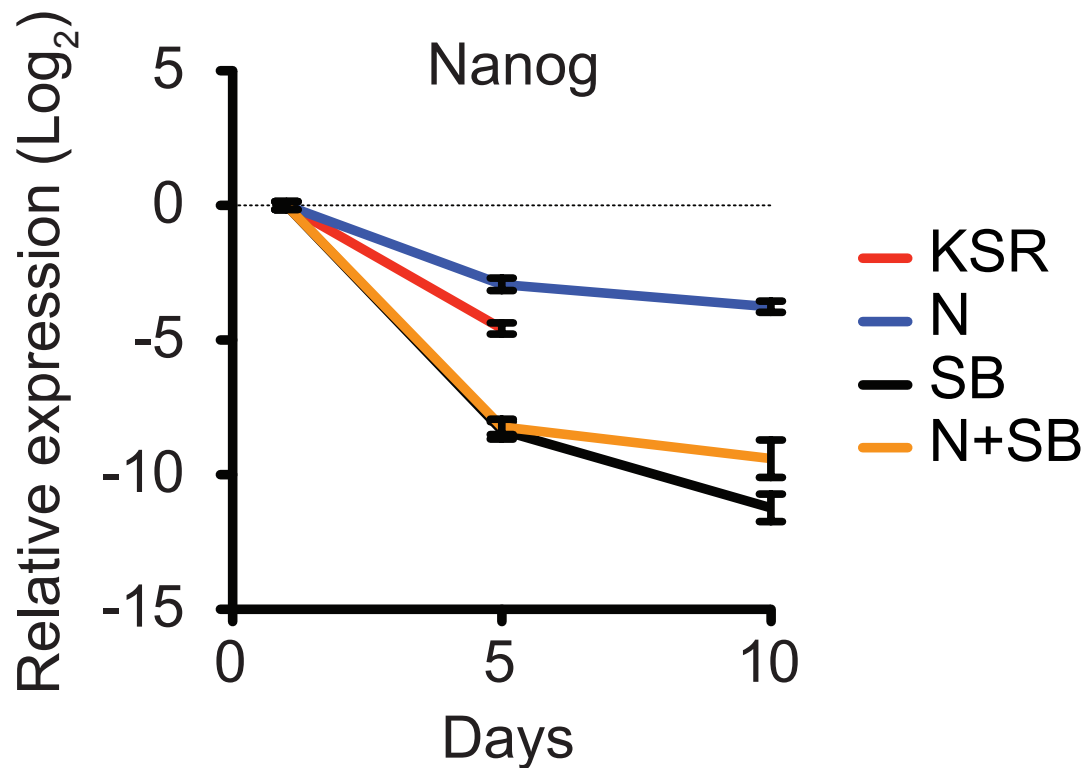
KSR
+ Noggin
+ SB431542



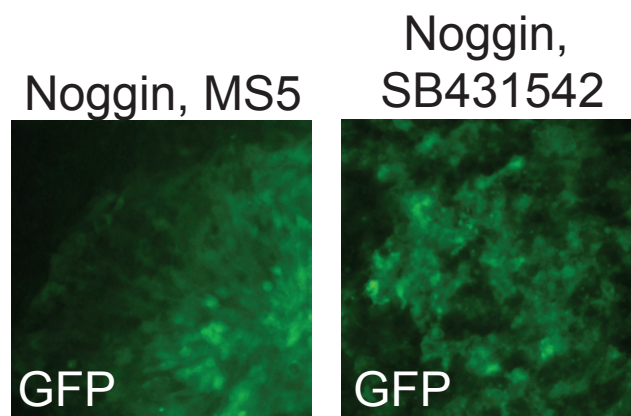
Supplemental Figure 1 - Nuclear localization of SMAD4 diminishes when hESC cells are treated with Noggin and SB431542 for 24 hours. A proportion of SMAD4 redistributes to a perinuclear localization resulting in a less defined cytoplasmic-to-nuclear border.



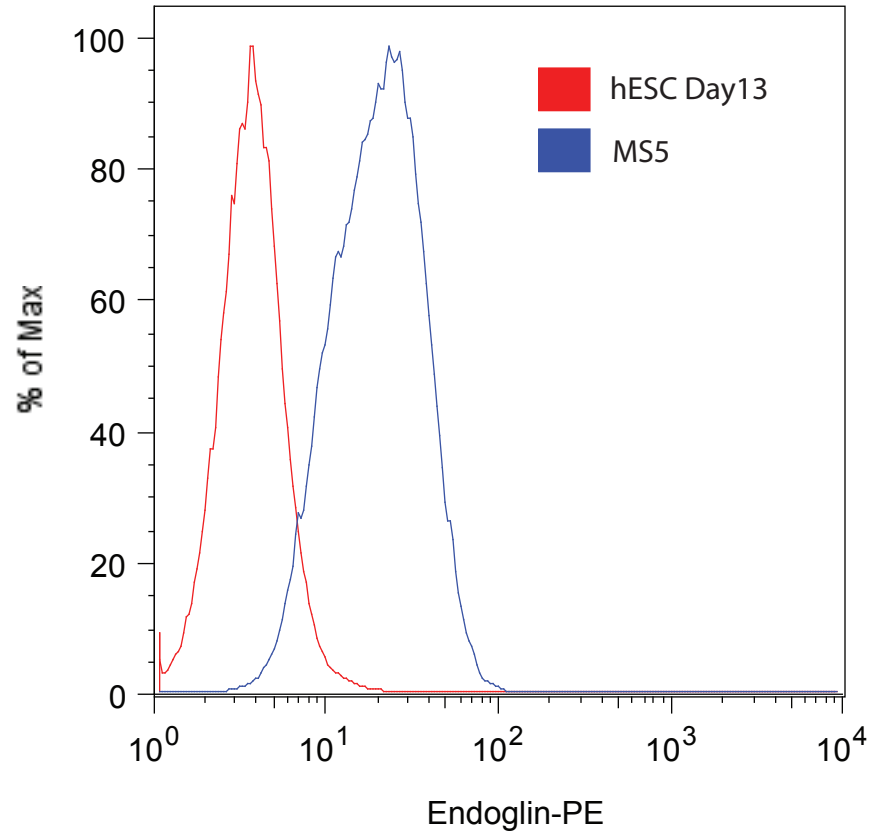
Supplemental Figure 2 - Model of potential mechanisms that contribute to the synergistic action of Noggin and SB431542. These include destabilizing the TGF β /activin- and Nanog-mediated pluripotency network, suppression of BMP induced differentiation towards trophoblast lineage, suppression of mes-/endodermal fates by inhibiting endogenous activin and nodal signals, and promoting neuralization of primitive ectoderm by BMP inhibition.



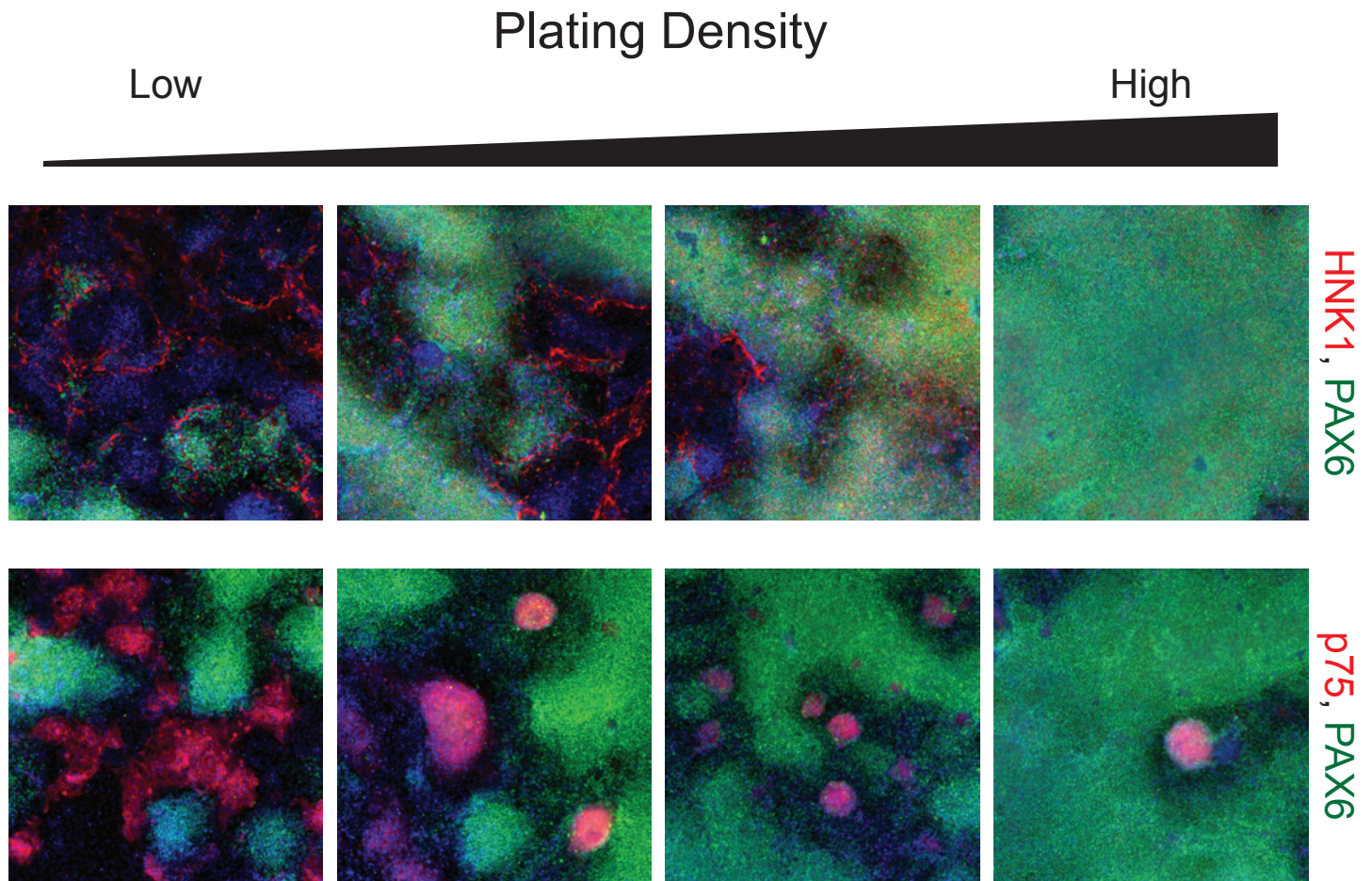
Supplementary Figure 3 - Nanog Real-Time gene expression. hESC treated with knock-out serum (KSR), Noggin (N), SB431542 (SB), or Noggin and SB431542 (N+SB) in KSR were examined for Nanog expression. The most dramatic downregulation was observed with the addition of SB431542. The error bars represent S.E.M.



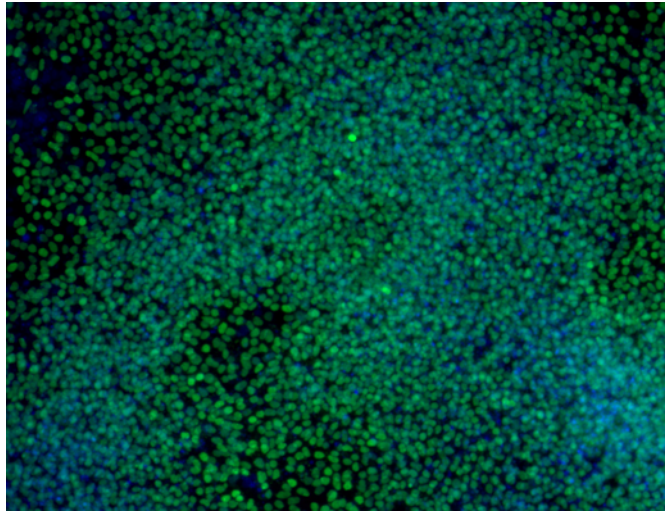
Supplemental Figure 4 - GFP expression of HES5-GFP BAC reporter hESC line. GFP can be observed under both conditions for neural induction (Noggin on MS5s or Noggin with SB431542) at day 13 of differentiation.



Supplemental Figure 5 - Endoglin (CD105) expression on MS5 feeder cells. MS5 cells used to differentiate hESC are uniformly positive for Endoglin (CD105) expression based on FACS analysis compared to hESC differentiated on day 13 using combined SMAD suppression. Endoglin expression was used to discriminate and remove MS5 cells from HES5-BAC hESC cells during the neural differentiation.



Supplementary Figure 6 - Plating density influences PNS vs CNS cell generation. Initial hESC plating density determines the ratio of neural-crest (HNK1,p75; red) to neural tissue (PAX6; green) present at day 11 of differentiation, with higher densities favoring neural differentiation.



Supplemental Figure 7 - Combined SMAD inhibition may only be required for the first 5 days of neural-induction. Homogeneous PAX6 expression could be observed on day 11 when SB431542 and Noggin, supplemented in the media, were withdrawn on day 5.